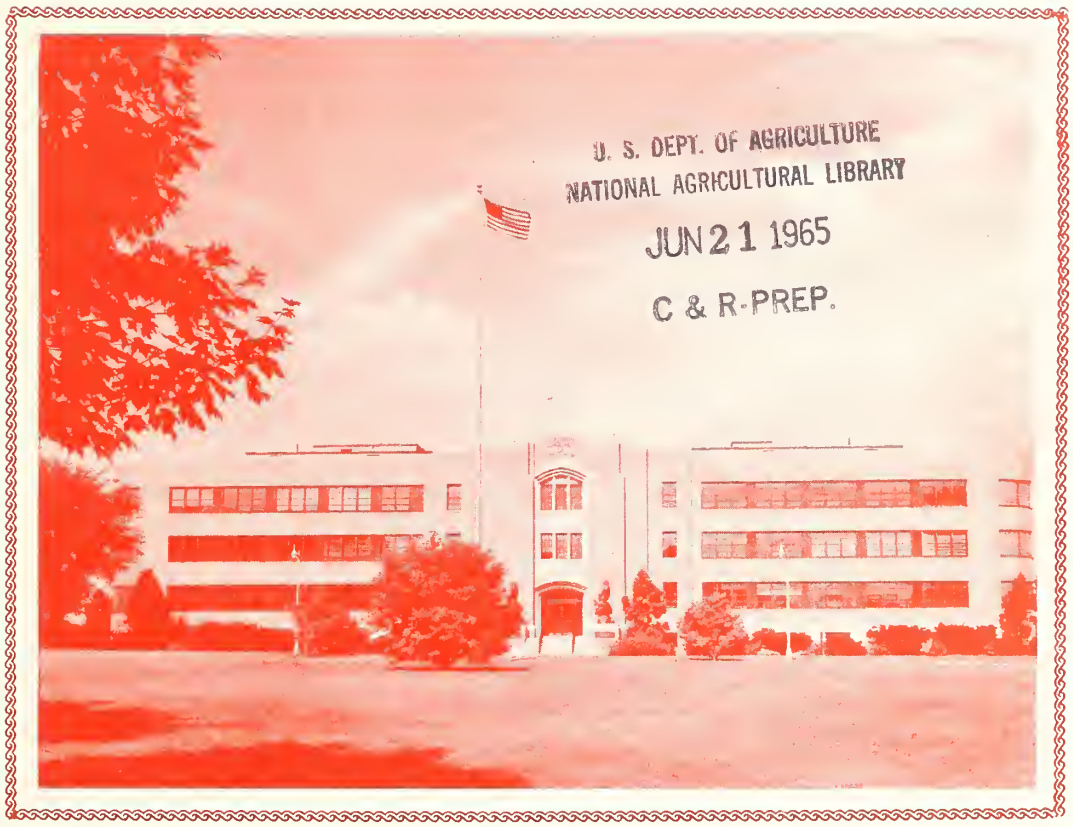
Report of Proceedings

Eastern Experiment Station Collaborators' Conference on POST-HARVEST PHYSIOLOGY



October 27 & 28, 1964

EASTERN UTILIZATION RESEARCH & DEVELOPMENT DIVISION
AGRICULTURAL RESEARCH SERVICE
U.S. DEPARTMENT OF AGRICULTURE
PHILADELPHIA, PENNA. 19118

Report of Proceedings

Eastern Experiment Station

Collaborators' Conference on

POST-HARVEST PHYSIOLOGY

October 27 and 28, 1964

Held at the

Eastern Utilization Research and Development Division

Agricultural Research Service

U. S. Department of Agriculture

Philadelphia, Pennsylvania 19118

This conference was attended by representatives of the State Agricultural Experiment Stations, universities, the Research Institute for Advanced Studies, the Republic of South Africa, and the U. S. Department of Agriculture. (Names and addresses of the conferees are listed at the end of this report.)

EASTERN EXPERIMENT STATION COLLABORATORS' CONFERENCE

ON POST-HARVEST PHYSIOLOGY

October 27 and 28, 1964

Program and Contents

INTRODUCTORY REMARKS

by

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Dr. Wells opened the meeting by welcoming the conferees. He pointed out that we meet each year with the Eastern Experiment Station Directors at their spring meeting and select a topic of mutual interest for our Collaborators' Conference for that year. This year the topic of Post-Harvest Physiology was selected. These Collaborators' Conferences were started in the Eastern Region about 1947. A number of the conferences held here in the past have continued to meet annually at various locations. For example, Potato Utilization Conferences are held annually since the first meeting here, as well as the Tobacco Chemists and Milk Concentrates.

Milk Concentrates is the latest of this series that has continued to meet annually after holding the first two conferences here and will return here next year for their 7th conference, which will also constitute our 1965 Collaborators' Conference.

Dr. Wells then introduced Mr. Wilbur T. Pentzer, Director of the Market Quality Reasearch Division of ARS, who served as moderator for most of the sessions during the first day. Mr. Pentzer pointed out that post-harvest physiology is a field of great interest to the Market Quality Research Division and one in which they are making considerable progress. When practical problems are encountered, i.e., scald, pits, etc., it points up the need for fundamental study of the principles involved. This is now a well recognized field of plant physiology, a subject which we can discuss and share the results of our findings.

PROBLEMS AND PROGRESS IN POST-HARVEST PHYSIOLOGY RESEARCH

by
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In the last decade a great deal of progress was made in physiological studies of harvested fruit due to the introduction of new methodology and to an experimental approach which took advantage of discoveries in general biochemistry. Fruit physiologists made use of their favorable experimental materials and compared metabolism at different levels of organization including the intact organ, the tissue slice, the particulate system and the soluble components.

The information obtained from the whole fruit subjected to controlled conditions led to the conclusion that respiratory metabolism is the chief process during the postharvest stage. Two patterns of respiration may be distinguished: (A) an upsurge followed by a decline and referred to as the "climacteric," and (B) continuous decline or gradual rise unaccompanied by sudden changes. Color changes are associated with both (A) and (B) while marked chemical changes in the flesh characterize fruits of pattern (A). The suggestion was made that the "climacteric" term should not be applied to any respiratory rise but to those cases in which the ripening process is involved.

A distinction was drawn between the two classes of fruits in terms of response to externally applied ethylene. In the climacteric fruits this unsaturated hydrocarbon brings about a shift in time of the onset of the rise but causes no increase in the gas exchange values at the peak when applied in a wide range of concentration. On the other hand, in non-climacteric fruits the rate of respiration is a function of ethylene concentration. The question was discussed whether the induced stimulation by exogenous ethylene has any bearing or may serve to explain the role of endogenously produced ethylene. Gas chromatographic studies have shown that minute but measurable quantities of ethylene accumulate in fruits during maturation. In some immature fruit the internal concentrations appeared to be of the order of the stimulatory levels, but caused little or no response. Apparently the sensitivity to ethylene increases with advancing maturity.

Whether ethylene is or is not the direct causal agent in inducing the climacteric rise it is relevant to examine the metabolic changes during this critical stage in the life of the fruit. Conceivably an understanding of the respiratory mechanism may lead to clues as to the mode of action of ethylene. For this purpose the oxidative reactions in tissue slices were examined. A striking stimulation of oxygen uptake by 2, 4-dinitrophenol (DNP) was observed in slices from preclimacteric fruit but not from climacteric peak. It appeared, therefore, that the phosphorylative machinery could be implicated in the changes preceding senescence. Phosphate esterification was studied with the isolated cytoplasmic particles of the avocado fruit. The rate of uptake of inorganic phosphate increased with the rise in respiration though the rate of oxidation decreased, at least with pyruvate and α-ketoglutarate as substrate. Consequently a high P/O ratio was obtained with mitochondria from climacteric peak as compared with those from preclimacteric fruit. The response to DNP was

dependent on preparative procedure. When precautions were taken to remove aggregates of particles DNP at proper concentration brought about uncoupling in both stages of maturation. By the use of DNP in combination with oligomycin it was possible to compare phosphorylation at the electron transport level (with succinate as substrate)with substrate level phosphorylation (oxidation of α-keto-glutarate to succinyl-CoA). The DNP stimulated adenosinetriphosphatase might be responsible for the relatively low phosphorylative efficiencies observed in some studies.

The role of phosphorylation in fruit respiration was attacked also in tissue slices through the use of single inhibitors or combinations of two or more inhibitors of the cytochrome oxidase enzymes or of other sites in the electron transfer chain. A striking aspect of this study was the stimulation of respiration by certain inhibitors. When azide or amytal was used singly the rate of oxidation increased and phosphate esterification was not suppressed. Carbon dioxide evolution was affected differentially from oxygen uptake. The idea was advanced that both phosphorylative and non-phosphorylative electron transfer chains operate in avocado fruit metabolism and that these chains are linked in a fashion that can make maximum use of one branch when another is blocked. In climacteric fruit more pathways are available for a greater variety of processes which become activated with ripening. The period immediately preceding senescence and breakdown might be viewed, therefore, as a phase of considerable synthetic activity.

THE BIOCHEMISTRY OF SENESCENCE

by
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Perhaps the most straightforward way to deal with the problem of senescence and death in plants is to adopt the hypothesis that senescence is simply the final phase of development and differentiation. Senescence therefore should be subject to the same kind of controls as all other phases of development.

As observed by Molisch (1928) the sudden death of annuals and biennials is not inevitable. It is brought on by flowering, fruiting and the production of seed and in many instances can be delayed indefinitely by preventing flowering. Under optimum conditions the century plant, Agave Americana, flowers, fruits and dies after eight to ten years. Under unfavorable conditions vegetative growth may continue up to 100 years before development of the inflorescence. The whole plant dies the season it flowers. Many annuals can be caused to live for several years by removing the flower buds as quickly as they form. Molisch believed that the formation of the flower and fruit depleted the vegetative organs of their reserves thereby causing exhaustion death of the plant. Leopold et al. (1959) have shown that this explanation is inadequate. Removal of the flowers delays senescence in both pistillate and staminate spinach plants. The size of the flower parts of the staminate plants is small compared to the bulk of the vegetative organs. Exhaustion can not therefore be a significant factor. Furthermore, removal of the filled but unripe fruit from the

pistillate plants delays senescence of the vegetative part of the plant. These results are best explained by supposing that the reproductive organs produce a signal of gradually increasing intensity (a hormone or hormone balance) which causes the vegetative organs to senesce.

The discovery of a number of substances which delay the normal yellowing and loss of protein of detached leaves greatly strengthens the hypothesis that senescence is under deliberate control. Kinetin retards the loss of protein and delays the yellowing of detached Xanthium leaves (Richmond and Lang, 1957). Benzimidazole has a similar effect on detached wheat leaves (Person et al., 1957). Normal autumnal yellowing and leaf-fall of deciduous trees is delayed by spraying the branches with gibberellic acid (Brian et al., 1950). Suitable formulations of either 2, 4-D or 2, 4, 5-T will delay senescence in both attached and detached leaves of a number of temperate deciduous species (Osborne, 1959). The treated leaves retain not only their green color but also their capacity for photosynthesis, RNA synthesis and protein synthesis.

N^6-benzyladeine delays the senescence of many harvested crop plants. Treatment results in a decrease in respiration, retention of chlorophyll, decreased desiccation and the retention of an increased proportion of the total phosphorus in an organic form (MacLean, D.C. and R.R. Dedolph, 1964). Treatment of attached bean leaves with N^6-bensyladeine stimulates the growth of the treated leaves and hastens the senescence of the adjacent untreated leaves, (Leopold and Kawase, 1964).

These examples of the modification of leaf senescence by the application of known hormones can leave little doubt that the mature leaf cells are still subject to the normal metabolic controls. It is not possible to specify from these data what reactions-or loss of reactions-lead to senescence. Continuing RNA synthesis and protein synthesis are necessary to prevent senescence. This is hardly surprising. We should however take interest in the fact that the agents which delay senescence also enhance RNA and protein synthesis. Is senescence the result of a hormone balance which will not allow the transcription and translation of the information needed to keep the cellular machinery in good repair?

Closely related to the problem of leaf senescence is the problem of abscission of various plant organs. The specialized cells of the abscission zone develop a separation layer as a result of hydrolytic processes in the cell walls. More than hydrolytic processes is involved. Abscission is temperature sensitive, requires oxygen, is inhibited by respiratory poisons, and is associated with a drop in endogenous auxins. Napthalene acetic acid, 4-chlorophenoxyacetic acid, 2, 4-D and related compounds are frequently used to retard abscission. A wide variety of compounds will induce abscission, particularly defoliation. The naturally occurring agents which induce or accelerate abscission are of great interest. These include ethylene, abscisin I (Liu and Carns, 1961) which has not been characterized, abscisin II (Ohkuma et al., 1963) which is acidic and has the molecular formula $C_{15}H_{20}O_4$, an unidentified substance in senescent Coleus petioles (Jacobs et al. 1962) and gibberellic acid (Ohkuma et al., 1963). Abscisin II is isolated from young cotton fruit and as little as 0.01 μg per abscission zone is effective on the excised cotyledonary nodes of 14 day old cotton seedlings. Gibberellic acid also accelerates abscission in the cotton explant test. This is reminiscent of the gibberellic acid induced production of hydrolytic

enzymes in barley aleurone layers. It remains to be shown whether new RNA and new protein are synthesized in the cotton explant abscission layers in response to added abscisin and gibberellic acid. These syntheses might be expected because respiratory poisons prevent abscission.

To summarize, the data all but establish that in the various kinds of senescence characteristic of plants, nothing is left to chance. The dramatic changes which are associated with senescence occur in response to definite signals. Cellular senescence might result from the failure, provided by a given signal, to produce the m-RNA necessary to keep the cell in good repair or it might result from the formation, at a given signal, of m-RNA which would direct the synthesis of degradative enzymes. For example, an increase in the m-RNA specific for RNA-ase would initiate the general degradation of senescence. In either event the next questions are "what triggers the suicidal impulse?" and "exactly how is it triggered?"

The activities of several enzymes of isolated barley endosperm increase markedly in response to added gibberellic acid. In the normal, intact germinating seed, evocation of these same enzymatic activities in the endosperm is caused by the embryo which is known to produce gibberellic acid. We have then an example of hormonally regulated enzymatic activity and one with which it is particularly convenient to work since the principle enzyme involved is α-amylase.

Simple dissection experiments have shown that only the aleurone layer cells are capable of respiration and amino acid incorporation. The Qo_2 ($\mu l.\ 0_2.\ hr^{-1}.\ 100$ mg frest wt^{-1}) for the aleurone layers at 25° is 15-30. The aleurone layers consist of a single cell type derived from the triple fusion nucleus. Aside from the possibility that a layer of living cells surrounding the dead starchy endosperm may provide protection against attack by microorganisms, the only obvious function of the aleurone cells is to produce and secrete hydrolytic enzymes for the digestion of the reserves of the dead starchy endosperm cells. It is a delightful nicety that the key to these reserves is kept by the embryo - the only tissue capable of growth.

The development of α-amylase activity by isolated aleurone layers of barley endosperm is completely dependent upon added gibberellic acid and is a result of the de novo synthesis of the α-amylase molecule. The synthesis of α-amylase and of other heat-stable proteins is prevented by actinomycin D. It is therefore postulated that gibberellic acid controls the synthesis of α-amylase and of other heat-stable proteins in aleurone cells by causing the production of specific messenger RNA's.

THE REPEATING UNIT OF PLANT MITOCHONDRIAL MEMBRANES

by
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The mitochondrion is known to be the major energy transducer of non-photosynthetic cells. Description of the mechanism of the transduction process has been extremely difficult. However, understanding of this process has advanced significantly in recent years. One of the most important advances has been the recognition that function of the transducer components (components of respiratory electron transport and oxidative phosphorylation) is dependent, in part, upon their association in a highly organized structural complex. With this advance has also come recognition that some reactions in transduction occur in a nonaqueous lipid milieu.

Fractionation of animal mitochondria and reconstitution of a functional unit from the various fractions, led to the concept that a recurring elementary unit of structure and function exists within the mitochondrion. The elementary unit would, thus, be the smallest entity that could be derived from the mitochondrion retaining the functions of electron transport and oxidative phosphorylation. Overall structure and function of the mitochondrion would be an expression of the properties characteristic of the elementary unit.

The purpose of this discussion is to present evidence supporting the concept that such a recurring elementary unit of structure and function exists in plant mitochondria. It has been demonstrated in this and other laboratories that plant mitochondria contain a number of cytochromes, some of which are very similar to their animal counterparts, i.e., cytochromes c, a and a_3. They do not contain cytochrome c_1. There are three b-type cytochromes, and an extraordinary a-type cytochrome which are not present in animal mitochondria. These cytochromes are assumed to be functional in electron transport in plants; perhaps certain ones are involved in the cyanide-resistant respiration which is characteristic of many plant tissues. Their characteristic absorption bands at low temperature (-196°C) were used to evaluate the capabilities of submitochondrial fractions in the present study.

Mitochondria from white sweetpotatoes were suspended in sucrose and sonicated for 20 min. The suspension was then fractionated by centrifugation. Pellets were resuspended in sucrose. All fractions, including the high-speed supernatant (105,000 x g x hr) were found to contain a full complement of cytochromes in relative proportions similar to whole mitochondria. The various fractions also contained electron transport chain activities which were qualitatively similar to those of the whole mitochondrion. These experiments provide evidence that a population of particles of varying size, all containing the necessary apparatus for electron transport, in similar proportions, is obtained upon mechanical disruption of the mitochondria. The significance of these experiments lies in the fact that none of the particles contained less than a full complement of cytochromes. These components are apparently arranged in a structurally organized unit, in such a way that cleavage occurs more readily between adjacent units than between components of a given

unit. This work furnishes further evidence for a basic unit of structure and function in mitochondrial membranes, and moreover, that the unit of the plant system contains all those respiratory components peculiar to plant mitochondria. The problem of identifying the basic unit by techniques of electron microscopy has not been solved but is the subject of present work.

DEVELOPMENTS ON POST-HARVEST USE OF CONTROLLED OR MODIFIED ATMOSPHERES FOR QUALITY RETENTION OF HORTICULTURAL CROPS

by
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Many interesting developments have accompained the great expansion in use of controlled-atmosphere CA storage for apples in the U.S.A. While commercial use of CA is primarily for apples, exploratory research on its feasibility for other crops is extensive.

CA had its beginning in 1920 when Kidd and West in Great Britain reported that they kept apples 8 months in 14% CO_2 and 8% O_2. In 1940 the first commercial CA facility was built in New York. Growth was slow until the last 5 years when it became spectacular. In 1964, CAcapacity will reach 12.5 to 13 million bushels with all production areas in the U.S. building facilities.

Overseas, Fidler in England and Duvekot in Holland state that with increased fruit acreage, there is expansion in CA capacity. Most new CA storages are using dry lime for CO_2 scrubbing. Landfald in Norway, Ginsburg in South Africa, and Martin in Australia all report considerable research on CA for fruit but commercial application is slow in starting. The important late varieties in these countries have a long life in refrigerated air storage if handled properly. There is no commercial use of CA for vegetables or flowers in any of these countries.

The most noteworthy development in CA equipment is the Tectrol atmosphere generator of the Whirlpool Corp., in which production of desired O_2 and CO_2 levels are externally produced and not dependent on living activities of the fruit. Commercial tests with CA generators for apples started in 1961 and Tectrol capacity since has grown to 3.2 million bushels in 1964 or nearly 30% of CA capacity. Research workers have compared keeping quality of apples from generated CA rooms and conventional CA and found no adverse effects of the artificially generated atmosphere. Where the only difference between two CA rooms is the method of maintaining the atmosphere, the condition of matched lots of fruit should be similar. The various advantages of atmosphere generators were discussed and the disadvantage of higher cost was mentioned. Some experience in using artificially generated CA for sweet cherries, pears, apricots, berries, citrus, pineapples, peaches, plums, lettuce, tomatoes, beans, cucumbers and sweet corn has been reported by the Whirlpool Corp.

Scrubbers to remove CO_2 from CA rooms are of many types. Caustic scrubbers

are in wide use for the 30° F. CA rooms of the Northwest and water scrubbers are widely used in the East. Many new CA rooms are employing hydrated lime CO_2 scrubbers because of their simplicity, efficiency and economy. Fifty-pound kraft bags of dry lime are placed in a gas-tight box outside the CA room but connected to it with inlet and outlet pipes. Approximately 1 to 1.5 lbs. of lime per bushel of fruit is adequate for 6 months in refrigerated storage. The effectiveness of small hydrated lime inserts for removing CO_2 from film-lined boxes or consumer packages was discussed. Lime inserts are now being used commercially to prevent brown core (CO_2 injury) of pears in sealed polyethylene-lined boxes.

Commercial usage of film packaging materials to develop beneficial modified atmospheres for horticultural crops was discussed. Currently low-density polyethylene liners are used to retard deterioration of pears, Golden Delicious apples, sweet cherries, lettuce for overseas shipments, greenhouse cucumbers and French endive, and numerous other consumer-packaged commodities. However, even with the wisest choice of packaging materials and storage conditions, variation of fruit respiration from lot to lot may occasionally lead to trouble from excessive CO_2 or too low 0_2 when packages are sealed. Thus packaging to obtain the benefits of modified atmosphere will always be more of a gamble than CA storage.

Currently there is little or no use of CA or modified atmosphere for holding cut flowers, although in Europe there is considerable interest in pressurized gas packaging in film bags for narcissus, iris, freesias and carnations. CA experiments with daffodils by Asen, Parsons, and Stuart of the U.S.D.A. were quite successful. Fully open daffodils stored at 40° F. in 100% N_2 for as long as 3 weeks had a display life on removal as long as freshly cut flowers. This atmosphere was more effective than 99% N_2 with 1% O_2. Unpublished CA experiments of Uota in California with cut roses, using low O_2 or 1% or less and storage at 32° or 59° have shown promise of retarding opening. Asen and Lieberman have reported that treatment of cut roses with 0.25% ethylene oxide will effectively retard maturation. The critical dosage range and the hazards of using this gas may prevent commercial application.

(The complete paper with citations covering developments on the use of controlled atmospheres for quality retention is available from the author. The following list of 100 selected references provides research background on a wide variety of horticultural crops).

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PRESENT STATUS OF APPLE SCALD RESEARCH

by
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One of the best known and perhaps the most widely studied physiological disorder of apples is apple scald, sometimes known as storage or superficial scald. Scald symptoms sometime appear in the late storage period but most of the injury does not become evident until the fruit is removed to a warmer temperature. The surface of the fruit turns brown affecting hypodermal and in severe cases some of the cortical tissue of the fruit. Generally the apple remains firm and unless there is a secondary infection by rot organisms the rest of the fruit is not affected. Sizable economic losses are due to the unacceptable appearance of the fruit. Consumers, believing that decay and/or over-maturity is involved, react strongly against purchasing scalded fruit.

Many workers have studied this problem during the last 60 years but the exact nature of this physiological disorder is still unknown. An old method of controlling apple scald involved the use of oiled paper. This method was not very effective and had a number of disadvantages. At the present time there are two materials recommended for the control of apple scald - "Stop Scald" (Ethoxyquin - 6 ethoxy, 1, 2 - dihydro - 2, 2, 4 - tri-methyl quinoline) and diphenylamine (DPA). In our experience "Stop Scald" was less effective of the two materials. (DPA) may be used as a spray or a dip at a 1000 to 2000 ppm concentration and may be applied just before or after harvest. The best control has been obtained by post-harvest dips. There are some difficulties in using this material and if all directions are not followed injury may occur on some varieties. In the occasional years when scald is unusually severe neither of the treating agents produces satisfactory control.

A number of investigators are conducting scald research of two different types. One involves practical application of control measures including various formulations, rates and time of application. The other is directed to an understanding of the causal agent or agents and the plant processes involved.

A number of theories have been proposed to explain storage scald. So far none have provided a complete explanation of all the conditions affecting scald development. Work at the University of Rhode Island has included relationship of time of harvest and duration time of storage to scald. Results show that time of picking, or perhaps more accurately fruit maturity stage, is a determining factor in scald development. Data showed that fruit picked very early does not scald, later-picked fruit increased in scald susceptibility reaching a peak near a "normal" commercial harvest, and later-picked fruit was less susceptible to scald. Red colored fruit was less susceptible to scald but coloration was not as important as time of harvest in influencing scald development.

A considerable amount of work was done during the last 10 years to see if the cuticle influenced scald formation. In general cuticle does appear to influence scald severity. Removal or decrease in cuticle reduced scald development. The exact role or function of cuticle is still not determined. Mineral oil and CO_2 control of

storage scald are frequently very effective, but due to a number of disadvantages and inconsistancy of results neither of the methods is commercially acceptable.

Preliminary research results indicate that there may be another effective material for scald control. This is a N-dimethyl amino succinamic acid manufactured by the Nagatuck Chemical Division, United States Rubber. At the present time this material is marketed as B-nine for use as a growth regulator on ornamental plants. Experimental material is still coded as B-995. When this material is applied as a spray on apple trees it reduces the size of fruit and appears to control scald development. Only one year's results are available and more work is in progress to evaluate this material for scald control.

Although we do have ways and means of controlling or at least decreasing severity of scald, its causal agent is still unknown. Even the developing process of scald is not clearly understood or explained. Apparently the only possible conclusion at the present time is that we need more research. Research should be concentrated on the basic principles and processes involved in scald development.

POST-SLAUGHTER CHANGES IN MEAT

by
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In order to understand the biochemical changes which occur in meat after the death of the animal, it is necessary to first consider the reactions involved in muscle contraction. The energy for muscle contraction is derived from the splitting off of one PO_4 group from ATP. This reaction is catalyzed by myosin and may be abbreviated as:

$$ATP \rightarrow ADP + PO_4 + \text{energy}.$$

The ADP is returned to ATP by the Lohman reaction:

$$ATP + \text{creatine} \rightleftarrows P\text{-}C + ADP.$$

This reaction, which is very rapid and highly reversible, is so efficient that very little ADP can be found in living tissue. An alternate pathway for ATP production is the glycolytic cycle which may be briefly written:

$$\text{Glycogen} + 3ADP + PO_4 \rightarrow 2 \text{ lactic acid} + 3ATP.$$

In life the lactic acid is reconverted to glycogen in the liver and the cycle continues. After death the oxygen supply to the muscle ceases, so that the Lohman reaction cannot proceed and lactic acid, from glycolysis, builds up in the tissue until all the ATP is consumed, or until the pH falls so low as to inactivate the enzymes. In the meantime the actin and myosin in the sarcomeres combine to form a new protein polymer, actomyosin. This is a very insoluble protein and comparatively little is known about it.

When this occurs the animal is said to be in "rigor." At this time the muscle will be exceedingly tough, and the pH will usually be about 5.5 to 5.8. During subsequent aging the muscle will become more tender. The complete reason for this is unknown, but it has been attributed to a mixture of proteolysis, actomyosin dissociation, and ionic shifts.

Post-slaughter chemical changes are characterized by an increase in soluble N_2, an increase in inorganic phosphorus, and an increase in free fatty acids. Data were presented on these and related changes in tenderness, water-holding capacity, and juiciness.

Since the principal interest in these changes centers around the tenderness problem, future research must be devoted to a better understanding of the actomyosin complex and to ways to activate the natural cathepsins which are capable of considerable tenderization, if they could be utilized. Balls found that one kg. of meat contains cathepsins equal in proteolytic activity to more than 25 mg. of papain. The activities of several cations in controlling some of the enzyme reactions of living muscle are already known, and further work may lead to new explanations of tenderness. In this connection the role of Zn is of especial interest.

GENESIS AND BIOGENESIS OF ETHYLENE

by
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Although the striking physiological effects of ethylene on fruit ripening have been known for over 50 years and the fact that it is a normal product of metabolism has been known for over 30 years, the origin of ethylene in plant cells and its mode of action are still unknown. There is no known biochemical pathway to which formation of ethylene can be assigned and nothing is known about the specifics of its interaction in metabolism which results in the dramatic ripening effects observed.

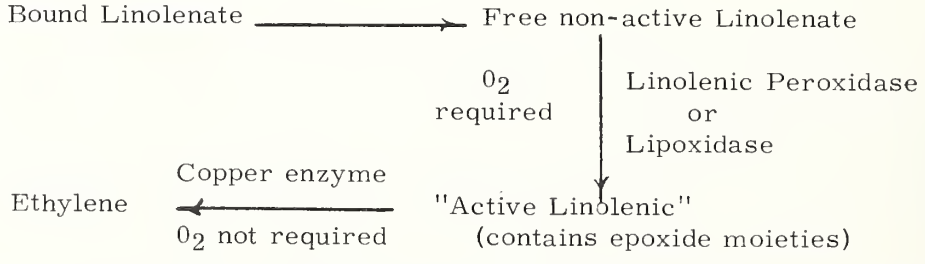
We have attempted to study ethylene biosynthesis in plants by isolating an in-vitro ethylene-forming system from apples by usual homogenization and cell fractionati on techniques. After some work along these lines, it soon became apparent that destruction of the cell also destroyed the ethylene-forming system. However, when cytoplasmic particles from apples were incubated with thiomalic acid or other α-thiol acids in acetate buffer at pH 4.5, a system capable of producing ethane was discovered. This system, which originally was thought to represent an enzymatic production of ethane, on closer scrutiny turned out to be a non-enzymatic production of ethane via the breakdown of linolenic acid. Cytoplasmic particles from apples were shown to contain a number of saturated and unsaturated fatty acids, but only the unsaturated acids were active in the ethane-forming system and linolenate was at least 3X more active than linoleate. Furthermore, this reaction was shown to depend on ferrous iron as a catalyst and it became clear that the cytoplasmic particles from apples supplied both linolenic acid and iron, which was reduced by the α-thiol acids. This left us with a non-enzymic model system which forms ethane. An important

prerequisite in this reaction is the proper "activation" of linolenic acid which essentially consist of the peroxidation of linolenate by oxygen. Once peroxidated, as evidenced by increased U. V. absorption spectra in the triene and diene regions (278, 268, 258 mμ and 233 mμ), ferrous ions could catalyze the production of ethane in an acetate buffer at pH 4.5. Substitution of cuprous ions for ferrous ions in the model system resulted in formation of ethylene. In this case, ascorbate was used as the reducing agent since α-thiol acids chelate copper ions.

The production of ethane and ethylene in the model systems can proceed in nitrogen atmospheres as well as in oxygen atmospheres, and in addition to ethane and ethylene also produce methane, propane, propylene, butane, isobutane and higher saturated and unsaturated homologues. In the presence of iron the saturated compounds predominate, while in the presence of copper the unsaturated hydrocarbons are produced in greater quantity. Some alcohols, ethers, and epoxides can also serve as substrates in the model system.

It is proposed that this reaction proceeds via a free radical mechanism in which either methyl or methylene free radicals are produced, depending on the nature of the metal catalyst; iron produces mainly methyl free radicals while copper produces mostly methylene free radicals. As these couple or abstract hydrogen from the medium ethane, ethylene or methane is produced, but by additional collision with each other higher homologues can be produced and these have been observed on chromatograms.

Analogy of this model system to the ethylene-forming system in the cells is suggested by effects of copper inhibiting agents and antioxidants on ethylene production by tissue slices. Evidence obtained by these means suggests that ethylene biosynthesis may proceed by a similar mechanism wherein peroxidated linolenate is broken down to ethylene by a copper containing enzyme which perhaps, because of greater specificity, can avoid production of the higher hydrocarbon homologues observed in the non-enzymic model system. The following scheme is suggested for the biosynthesis of ethylene in plants:



This work was done in collaboration with Dr. L. W. Mapson of the Low Temperature Research Station, Cambridge, England and with the technical assistance of A. T. Kunishi and D. A. Wardale.

ANTHOCYANIN FORMATION IN APPLE SKIN

by
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ABSTRACT

The environmental factors of primary importance in the formation of anthocyanin in apple skin are light and temperature. Examination of the time course of anthocyanin formation revealed two light dependent phases. During the first phase, called the induction period, which lasts for about 16 hours at 15°C, no anthocyanin is formed. The second phase, called the linear period, follows and is characterized by a period of linear formation of anthocyanin at constant light intensity.

Action spectra were determined for both the induction period and the linear period and they were found to be identical. An action maximum was found at about 650mμ and there was effectiveness of all wave lengths between 400 and 700mμ for promoting anthocyanin synthesis. Recent studies indicate that the photosynthetic system is the photoreceptor for this reaction. Phytochrome has also been shown to control anthocyanin formation in apple skin.

Temperature studies indicated that the maximum anthocyanin formation occurred at about 25°C during the light period and dropped rapidly below 20° or above 30°C. During dark incubation after exposing the apple skin to light, maximal anthocyanin formation occurred at 15°C.

The formation of anthocyanin in the apple skin is under the control of at least two photoreactions. The temperature for optimum synthesis in light and darkness are 25 and 15°C, respectively.

PRE - AND POST-HARVEST COLOR DEVELOPMENT IN APPLES

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Requirements for red color formation in the orchard were outlined. Night temperatures of 32° - 40°F and daytime temperatures of 70° - 75° F were found to be ideal for color formation. The limiting factor in getting red color in the orchard is usually lack of cold nights in the fall. To supplant lack of cold nights, attempts have been made to find a spray material that would induce red color formation. A technique for screening materials in the laboratory was described.

It was found that certain calcium salts would increase red color both in the laboratory and in the orchard. However, field experiments showed an average increase of only 13% red color with materials like calcium carbonate and calcium phosphate. The most promising material to date has been N-dimethylaminosuccinamic acid ("B-Nine"). In the laboratory, responses were seen even in June on dipped apples.

In the orchard, the number of "extra fancy" apples was increased more than with calcium salts.

All such materials must also be evaluated as to their effect on the physiology and keeping quality of the apples. Some materials like copper sulfate that gave a good coloring response resulted in leaf or fruit injury. Sometimes these injuries did not show up until the apples were in storage. The materials that are finally judged promising have no adverse effect. Apples sprayed with N-dimethylaminosuccinamic acid were not only redder, but had a marked delay in the climacteric rise and were firmer both at harvest time and after storage. There may be adverse effects from this material that have not yet been found.

RESPIRATION OF SEEDS IN RELATION TO VIGOR

by
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ABSTRACT

Studies with corn have shown that respiration rates during the early phases of germination are highly correlated with subsequent seedling growth. Pre-sowing treatments with gamma-irradiation (0 - 80 Krads), heat (0-4 days at 45°C.) and freezing temperatures (0 - 2 hrs at -15° C., immature seed only) were employed to provide a range of vigor. The treatments varied in severity from those which could not be detected by laboratory germination tests to those which markedly inhibited seedling growth, but did not cause much loss in viability. Respiration was measured manometrically on samples ranging from 1 - 25 seeds per flask at various times after planting and was expressed as $Q0_2$ ($\mu l0_2$/hr/seed). After the $Q0_2$ determinations, the seeds were replanted in paper germination towels, incubated in darkness at 25° C., and the root and shoot lengths measured several days later. Treatments severe enough to cause seed injury also inhibited respiration, while less severe treatments had no effect on respiration. In the less severe heat-treatments, respiration proved to be a more sensitive measure of seed injury than the germination tests, when the two were compared with rates of seedling emergence in the field. When both the speed and the predictability of the test are considered, the period between 4-6 hours after planting appears to be the best time to measure respiration. Measurements in an atmosphere of pure oxygen sometimes improved the correlations between $Q0_2$ values and seedling growth, but exposures of more than 4 hours to an oxygen atmosphere inhibited subsequent growth. Even the most mild heat-treatments caused a marked inhibition in respiration during the first couple of hours after planting. This inhibition was out of all proportion to the extent of the injury. That inhibition of $Q0_2$ values was truely an effect on respiration, rather than due to a physical release of gas from the seeds during wetting, was confirmed by the determination of $QC0_2$ values.

Preliminary studies with lima beans have also shown high correlations between respiration and growth, particularly when respiration was measured shortly after the start of inhibition. One-hour cold treatments applied at the start of germination

caused severe seed injury and markedly inhibited respiration, but these effects could be mitigated by pre-treatments with warm water. The reduction in respiration rates was generally proportional to the degree of injury sustained in the various combinations of treatments and therefore served as a rapid indication of the extent of the injury.

The above results indicate that measurements of respiration may provide a rapid and sensitive method for detecting a variety of kinds of seed injury, and that the method may be applicable to a number of different kinds of seeds. They also suggest that measurements of respiration might be used for evaluating seed vigor. Studies on the nature of the relationship between respiration and growth and on the effects of environmental conditions (during and after the $Q0_2$ measurements) on the correlations are in progress.

CURRENT SOUTH AFRICAN COLD STORAGE PROBLEMS IN DECIDUOUS FRUIT

by
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I wish to take this opportunity of thanking Dr. P. A. Wells for inviting my colleague J. M. Bester and myself to this conference. It is also an opportune moment for me to convey my very sincere thanks to the U.S.D.A.'s Fruit Research Organizations, State Agricultural Research Institutes and Faculties of Horticulture at Universities for their time and wholehearted co-operation during the visit of the technical mission of Deciduous Fruit Board of the Republic of South Africa to the U.S.A.

There are many problems of mutual interest. I leave the U.S.A. having learned much. It was pleasing to find that during discussions we were able to contribute something towards the better understanding of certain of your problems.

Is York Spot a form of Bitter Pit?

York Spot is a disorder unknown in South Africa. A cursory examination tends to suggest that it resembles Bitter Pit. The following are some symptoms which are not true to the Bitter Pit pattern. Many spots are invariably found in the stem end sector of the apple, this is rarely found in Bitter Pit apples. Necrotic lesions in the cortex of most apples suffering from York Spot tend to penetrate to the core of the fruit, this is not found even in apples suffering with severe Bitter Pit. York Spot does not respond to foliar sprays of calcium salts. This treatment, which is practised in Australia, New Zealand, South Africa and certain European countries, reduces the incidence of Bitter Pit to a minimum. The York Imperial apple grown under South African conditions is not a Bitter Pit susceptible variety. (Ginsburg and Beyers E. 1963). This fact strengthens the case that York Spot is a disorder which is not akin to Bitter Pit. This statement must be regarded with reservation. The Winter Banana apple, grown under South African conditions, is extremely susceptible to Bitter Pit, yet this same variety grown in West Germany is most resistant to Bitter Pit. Environment plays a vital roll in the susceptibility to a disorder and this fact must be taken in consideration when assessing the significance of the lack of Bitter Pit in South African grown York Imperial apples.

Bitter Pit:

While Bitter Pit is generally not regarded as a serious disorder in the U.S.A., many apple growers claimed they suffered big losses due to Bitter Pit.

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* While not a scheduled speaker on our program, Dr. Ginsburg attended the Conference and his remarks on the current problems in cold storage of deciduous fruits in South Africa given to us during the Conference are reproduced here in full since we felt they would be of interest to those attending.

They showed great interest in the methods used to combat this disorder. Bitter Pit has been a major disorder in South Africa for many years resulting in considerable losses to apple growers. The 1961-62 apple season was very conducive to the development of Bitter Pit and serious complaints were received from the United Kingdom concerning the Bitter Pit in Delicious, White Winter Pearmain and Golden Delicious varieties. (Ginsburg 1962). The inclusion of calcium salt foliar sprays in the spray program since the 1962-63 season has reduced the incidence of Bitter Pit to a bare minimum, this includes the latent development of this disorder during the cold storage. Careful check is made to ensure that all Bitter Pit susceptible export varieties are given the calcium spray treatment.

The calcium salt may be applied either as a nitrate or chloride, 7 lb of calcium chloride or 8 lb of calcium nitrate together with a suitable spreader (1/2 pint of Teepol 410 or 3 fl. oz. of Triton B) is added to 100 gallons of water. The calcium salts may be mixed with other spray materials to be used e.g. Gusathion or Sevin. The calcium salt and spreader is always the last ingredient to be added to the tank. The first calcium spray is applied approximately ten weeks before harvesting and this is followed by a further two sprays at 14 day intervals (E. Beyers 1963).

The calcium salts have been found to have other interesting effects on quality. It renders the apples more resistant to fungal decay and bruising. The use of calcium chloride has resulted in reducing the incidence of superficial scald on Starking delicious apples. This factor further complicates the very complex subject of superficial scald so ably dealt with by V. G. Shutak at this conference.

Botrytis rot in grapes:

The development of rot in grapes during storage is a problem of much importance both in the U.S.A. and in South Africa. The fungi largely responsible for the decay the grapes in both countries is Botrytis cinerea. The present method of control is by means of sulphur dioxide fumigation. The risk of sulphur dioxide gas injury to the grapes has continually to be faced when this technique is used, irrespective of whether the South African or Californian method is used. An urgent need for a safer means for controlling Botrytis cinerea exists.

Tests both in the U.S. A. and South Africa have established that dibromotetra-chloro-ethane (D. B. T. C. E.) very effectively controls the development of Botrytis cinerea development in stored grapes. The major disadvantage of D. B. T. C. E. is that it is a lachrymator and creates unpleasant conditions during its application.

The South African approach to minimize the pungent nature of D. B. T. C. E. is to apply it just before lidding the box. The grape box is carefully lined with polyethylene (grade 150), a bottom woodwool pad is placed in the box and 10 lb of wrapped bunches of grapes are packed into the container, being adequately cushioned with woodwool. A top sheet of relatively thick paper impregnated with 0.1gm of D. B. T. C. E. is placed over the top of the grapes, which are then completely enveloped in the polyethylene liners prior to the lidding of the boxes. The polyethylene envelope ensures that a high relative humidity is maintained round the grapes so ensuring green stems and turgid berries while the D. B. T. C. E. counters Botrytis cinerea development. Tests conducted in South Africa on Barlinka grapes provided with top sheets containing 0.1

and 0.2gm of D. B. T. C. E. resulted in 2.6% and 1.3% fungal rot respectively after four months storage at 31°F. The incidence of rot after six months storage at 31°F was 3.6% and 7.3% respectively. The incidence of fungal rot for the untreated samples after 4 and 6 months storage was 36.4% and 88.0% respectively. (Ginsburg and F. N. Matthee 1964).

A considerable amount of work in this field has been undertaken by K. E. Nelson of the Department of Viticulture and Enology at the University of California at Davis and by J. W. Eckert and M. J. Kolbezen of the Department of Plant Pathology also of the University of Calfiornia but at Riverside. The Crown Zellerback group are cooperating on this project and the aim at present is to control the rate of liberation of the D. B. T. C. E. by confining it in an appropriate plastic envelope. This technique, if successful, would minimize the lachrymatory nature of the D. B. T. C. E.

Influence of newer packaging materials on fruit:

Modern technology has had its impact on fruit packaging. The wooden container is rapidly being replaced by fibre board containers. Most apples are today being packed in either cell or tray packs in fibre board cartons. The fibre board and paper pulp used for the interleaf trays have wet strength agents incorporated to allow these materials to withstand the high relative humidities which are maintained in cold stores. Synthetic formaldehyde based resins were once used on a large scale for imparting wet strength to fibre board used for fruit cartons. The first trays and fibre board cartons used for packing apples were made according to U. S. A. specifications. It was very soon found that apples packed in such containers developed lenticel injuries. This injury can be recognised as black spots centered on lenticels. The injury results from the death of tissue round and immediately below the lenticels. It was found that this injury was due to free formaldehyde liberated during the hydrolysis of the urea formaldehyde in the fibre board and the melamine formaldehyde synthetic resin in the moulded pulp interleaf tray. (Ginsburg, L and Koepen B. H. 1963). The swing in South Africa from wood to fibre board containers was sudden. This simplified diagnosing the causative factor responsible for lenticel spots found on the apples several days after packing. Work by Harold A. Schomer of the U. S. D. A. Wenatchee has confirmed these findings. In South Africa Kymene 557 a polyamide has replaced the formaldehyde basic resin as wet strength agents and no further trouble has been experienced. It is interesting to note that certain European countries are still using packaging material causing lenticel spotting in their tray packed apples.

Polystyrene Interleaf trays for apple packs and tainting.

The moulded paper pulp interleaf tray has done much to reduce bruising in apple packs. There are strong indications that this type of tray may soon be superceded by polystyrene interleaf trays. It is planned that more than three million cartons of apples will be packed in South Africa during 1965 using the polystyrene interleaf tray. This material provides even better cushioning properties than moulded paper pulp. It is a very attractive material and is competitive from a price angle.

Investigations in South Africa have clearly shown that this material, though odorless under normal conditions, may taint apples when held in close proximity to fruit. It has been shown that the taint is due to the presence of free styrene monomer(or

short chain styrene products) which may be present in the polystyrene. The initial analytical techniques employed were gas-phase chromatography, this has been replaced by ultra violet spectroscopy. (Ginsburg L. and Woods G. 1964). It has been shown that where trays are manufactured from polystryrene beads containing 0.1% and higher of free styrene monomer, apples packed in such trays may be expected to develop a taint. The higher the stryrene monomer content the more noticeable the taint. Polystyrene with 0.08% free styrene and less have not been found to taint apples. As a result of these findings a recommendation has been made that polystyrene beads used for the manufacture of interleaf apple trays should not contain more than 0.05% free styrene monomer.

RUSSETING

Russeting on South African apples and pears detracts from the appearance of the fruit. The causes for the different forms of russeting are not fully understood. The incidence of this disorder varies greatly from one season to another. It is clear that environmental factors play an important role in the development of russeting. Equally important is the role played by certain spray materials. Hansen at the "Institut fur lebensmittelfrischaltung" at Karbaruhe W. Germany has for several seasons been studying the effect of different spray materials on russeting on apples grown in Germany. It was interesting to find apples grown in the orchards in the Washington State to be free from russeting. A program of four sprays per season, together with a dry growing season but with a sufficiency of water for irrigation, are conditions envied by all apple growers. The presence of russet on apples grown in the East, where an extensive spray program has to be followed, is more in line with conditions met with in the main apple producing areas in South Africa.

Sevin was thoroughly tested as a measure against mealy bug which was taking on serious proportions in all pear varieties in South Africa. The results on the varieties under investigation were most promising, with the result that it was used for all varieties.

A Very unusual case of severe russeting developed in Kieffer pears during 1964 The condition was so severe that the pears were unsaleable for dessert purposes. After a study of the spray programs followed, the growers concluded that the Sevin spray was responsible for the russeting. Preliminary tests conducted by South African consultant chemists put forward a theory that the lenticels in Kieffer pears were high in iron content. The severe dark russet centered on the lenticels of the Kieffer pears was due to an interaction between the Sevin and the Iron in the lenticels.

It should be stressed that the Sevin sprays controlled the severe mealy bug infection very effectively on all the pear varieties. The onset of severe russeting was only found in the Kieffer pear variety.

This example stresses the need for careful evaluation of new spray materials. Varietal responses can sometimes lead to very surprising results.

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